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Morphological analyses of sperms causing severe male infertility with light microscopy, motile sperm organellar morphology examination and electron microscopy technics

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ABSTRACT

Aims: To evaluate abnormal sperm morphology by three different methods including light microscope, real-time motile sperm organelle morphology assessment at 8050x high magnification and electron microscope and to determine degree of effect on morphologic defects.

Methods: Patient cohort of this study was selected among males referring to Memorial Hospital assisted reproductive methods and genetics center and having morphological sperm defects causing severe male infertility such as megalohead, tail stump, globozoospermia and pinhead abnormalities. 20 patients having morphological sperm defects causing severe male infertility and 3 control patients. 4 patients with megalohead abnormality, 6 patients with globozoospermia, 8 patients with tail stump abnormality and 2 patients with total pinhead abnormality were included into study and 3 voluntary fertile males formed the control group

Results: In megalohead group, together with multi-flagellum structure, irregular and large sperm heads, pinhead sperm abnormalities have been found to be accompanied. In globozoospermia type I group, sperm head were observed to be round and lacking acrosome, while in type II group, the cone-shaped nucleus and a small amount of acrosome were observed. In tail-stump group, sperm with short and blunt-shaped tail were also found to have scattered microtubules and 9+0 structure rather than 9+2. In pinhead group, some sperm with the head were seen, even it was rare, and 9+2 microtubule structure was striking.

Conclusion: Beside the light and electron microscopic techniques, the motile sperm organelle examination can offer information about sperm quality in a simple and quick way and by the Intracytoplasmic Morphologically selected Sperm Injection (IMSI) developed through the use of sperm selected by this technique can contribute to the success of in vitro fertilization centers. We believe that sperm selection at high magnifications can increase invitro fertilization (IVF) pregnancy rates in patients with sperm morphology that causes severe male infertility.

Keywords: Male infertility, IMSI, severe sperm morphology

INTRODUCTION

Infertility which is described as inability to conceive after 1 years of unprotected intercourse affect about 15% of couples at the moment. The causes of infertility affect males, females and both genders equally about one third for each.[1] In addition to sperm count and motility, sperm morphological abnormalities are important among parameters causing male infertility.[2] Sperms of infertile males have prominent morphological alterations enough to change functional characteristics when compared to sperms of fertile males. Globozoopermia, pinhead, megalohead and stump tail are among the most important sperm morphological defects causing infertility.[3]

In the last two decades, despite lack of improvement in evaluation techniques for sperm concentration or motility, there are considerable advance is evaluation techniques for sperm morphology. Since the aim of morphological evaluation is to make in vivo and in vitro predictions about sperm function, a meticulous selection of criteria for evaluation is of great importance.[4] Currently, sperm morphology evaluated according to Kruger criteria is the most important parameter in detecting fertile and infertile male.[5] However, in most of the sperm abnormalities detected by using Kruger criteria minor defects may be overlooked. In addition, diagnosis by fixed and stained sperm samples at the same time may also be considered as another disadvantage. Thus, Bartoov et al.[6]

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have developed a novel morphological evaluation method in order to make a detailed real time morphological evaluation of motile sperm cells. By this method called as motile sperm organelle morphology examination (MSOME), 6 organelles of sperm (acrosome, nucleus, post-acrosomal lamina, neck, midpiece and axoneme) are examined as unstained and real-time by using invert light microscope under 6300x-8050x magnification. Thus, evaluation of sperm morphology during ICSI procedures became possible.

By these types of studies, evaluating and/or revealing histological structure of sperms causing severe male infertility will be possible. As it is known morphological sperm defects are among important reasons effecting success of centers for assisted reproductive methods. In this study, we aimed to compare sperms of fertile males with morphological sperm defects causing severe male infertility (Megalohead, Tail stump, Globozoospermiaa [Round head], Dense Pinhead abnormalities) by examining sperms under light microscope, MSOME and ultra-structurally by transmission electron microscope (TEM). Thus, with this study we aimed to provide better understanding of sperm morphology and consequently to contribute to assisted reproductive methods.

METHODS

This study is the scientific study of Ferhat Cengiz master's thesis named "Morphological analysis of sperms causing severe male infertility with light microscopy, motile sperm organellar morphology examination and electron microscopy technics", registered at the National Thesis Center with the number 267264, dated 19.07.2010. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. All patients signed the free and informed consent form.

Patient cohort of this study was selected among males referring to Memorial Hospital assisted reproductive methods and genetics center and having morphological sperm defects causing severe male infertility such as megalohead, tail stump, globozoospermia and pinhead abnormalities. 4 patients with megalohead abnormality, 6 patients with Globozoospermiaa, 8 patients with tail stump abnormality and 2 patients with total pinhead abnormality were included into our study and 3 voluntary fertile males formed the control group.

Evaluation of semen sample was performed immediately after the end of normal liquefaction period (20-30 minutes). Concentration, motility, and morphology parameters were evaluated and recorded into semen analysis pre-registration form. In addition to this, volume, agglutination, pH and if present, immature germ cells other than sperm, red blood cells, leucocytes and epithelial cells were evaluated. To assess sperm concentration, after liquefaction of semen the material was homogenized by pipetting it several times by using Pasteur pipette. 5-10 µl sperm obtained by micropipette was counted in Makler Counting Chamber (Makler® Counting Chamber - Israel) by using phase contrast microscope (Olympus CH 30, Tokyo, Japan) under 20x magnification and sperm count was determined as million per milliliter. Motility and progression evaluation was done by using light microscope (Olympus CH 30, Tokyo, Japan) under 20x magnification according to WHO criteria and percentage of total motility, progressive motility, slow progression, in situ motility and immotility was calculated by counting at least 100 sperms. Percentage value of motile sperms among 100 counted sperms was regarded as sperm total motility.

Semen sample liquidated after evaluation was homogenized by liquid broth (ALLgrad wash- Canada) in 1/1 ratio and was concentrated by centrifugation in 1000 rpm/min for 10 min (Heraus Megafuge 40- Germany). After removal of supernatant, it was homogenized by adding 0.25 ml liquid broth onto pellet. After obtaining 10 µl, a fine smear was prepared by dropping on a clean slide.

After dried in the air Spermac Stain (Spermac Stain-Fertipro-Belgium) procedure was applied. After drying, the sample was evaluated by light microscope under 100x magnification and by phase contrast microscope (Olympus Bx50, Tokyo, Japan) with immersion oil. At least 100 sperms were counted and small head, long head, tail abnormality, neck and mid piece abnormality, amorph sperm or sperm with multiple abnormalities and specific structural defects; megalohead, tail stump, Globozoospermia, pinhead abnormalities were recorded into the sperm morphology evaluation form as percentage and images of the findings were taken.

Fresh ejaculate was used as sperm source for MSOME. Sperm preparation appropriate for each sample was done. After preparation sperms were stored in 0,2 ml HTF solution as suspension at 37°C. For evaluation an amount that contains nearly 5000 sperm from the prepared semen sample was inseminated into 10 µl liquid broth droplet within 10% range prepared over sterile petri dish with glass base and covered with sterile paraffin oil. Sperm evaluation was done by Zeiss Axio Observer A1 invert microscope equipped with DIC objective and DIC condenser. For imaging 1.0 x camera adaptor and 1/3" Sony DxC-390P 3 CCD video attached to microscope and 19" Samsung LCD monitor were used. Total predicted magnification was calculated as 8050x. For general classification of semen samples MSOME criteria Bartoov's table was used. In MSOME 6 organelle (acrosome, nucleus, post-acrosomal lamina, neck, midpiece and axoneme) were examined and images of findings were taken.

Sperm samples were concentrated by centrifugation under 2700 rpm/min for 10 minutes for routine electron microscopes. The sample was fixed with 2.5% gluteraldehyde for 4-6 hours. To prevent disintegration of concentrated sperm in the following electron microscope follow up procedures it was embedded into 2% pure agar. Subsequently, follow up used for routine electron microscopic examinations was performed in Trakya University Medical Faculty Histology and Embryology department. To detect section region of the blocks semi-thin section were obtained (RMCMTx Ultramicrotom American) and stained by azure blue. Thin sections with 40-60 nm thickness from the determined areas were obtained, sections were stained by uranyl acetate and to increase contrast by Reynold's lead citrate stains. These sections were examined under Jeol 1010 electron microscope and images of findings were taken.

Statistical Analysis

In statistical analyses, we considered a p-value < 0.05 to be significant. We used Chi-square test for correlation analysis.

RESULTS

Control Group

In light microscope sperm heads were usually oval with 4.0-5.0 µm length and 2.5-3.5 µm width. Acrosomal region was well defined, consisting 40-70% of the head. Mid piece was straight in most of the sperms and its ratio relative to head length was 1.5-2.0 and it was axially attached to the head. Tails were straight, slender in the mid portion, uncoiled and nearly 40-50 µm long (**Figure 1a**). There were sperms with abnormal morphology along with sperms that were normal.

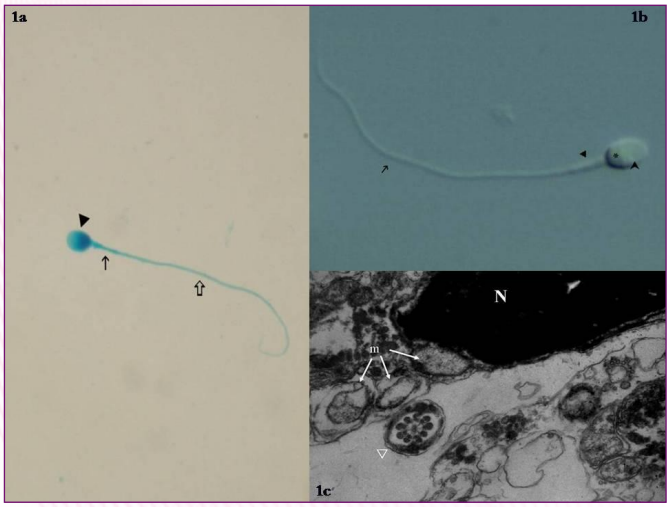

Figure 1. **1a.** Normal sperm appearance in control group: a well defined acrosomal region covering 50% of sperm head (◀). Mid-piece slender, long, axially attached to the head, contains no residual cytoplasm. (→). Straight, non-deformed, thin in mid portion, uncoiled and nearly 40–50 µm long tail is noticed (⇒). Spermac Stain, x1000 **1b.** Sperm appearance in control group: Small vacuoles less than 4% of sperm head is seen (▼). Nucleus is normal, symmetrical and oval (*). Neck axially attached to the head and doesn't contain residual cytoplasm(▲). A tail which straight, thin in the mid portion, uncoiled, having normal shape and dimension is noticed(→). MSOME, x8050 **1c.**Sperm appearance in control group: Nucleus is normal and includes mature chromatin (N), Mitochondria are normal and symmetrically located (m). In cross-sectional views tails containing microtubules that are in 9+2 design were noticed.(▽). Uranyl acetate-Lead sitrate, x50000

6 organelles of sperm (acrosome, nucleus, post-acrosomal lamina, neck, midpiece and tail) were examined by MSOME. Acrosomes were in the front side of head with proper structure and there was no vacuole within the nucleus. Neck was axially attached to the head and there was no residual cytoplasm. Mitochondria were located at neck properly. Tails were straight, slender in the mid portion, uncoiled and nearly 40-50 µm long. Nuclei were in proper shape with regular, symmetric and oval configuration (**Figure 1b**).

In electron microscope evaluation acrosomal cap region of the sperms were normal in shape, continuous and covering 50-60% of front part of the head with a dense content. Nuclei were normal in shape with mature chromatin. Axoneme structures in tails were in normal appearance. Fibrous sheath structures were appearing normal and as whole. In most of the microtubule structures that were observed 9+2 structure was present. Mitochondria were normal in shape and located symmetrically and regularly in both sides of the sperm neck (**Figure 1c**).

Megalohead Group

When evaluated according to Kruger criteria it was observed that most of the sperm heads were larger than normal size. In some patients large heads with regular structure in certain proportions were observed among sperms with normal size. Acrosomal cysts were seen in acrosomal region of some sperms. Within the sperms megalohead sperms along with pinhead sperms and tail problems were present. In some sperms there was irregular acrosomal regions covering sperm head and it was noteworthy that in some sperms mid piece wasn't axially attached to head. Residual cytoplasm and multiple tails with normal length were seen. Along with megalohead sperms double-head, double-tail sperms and morphologically indiscriminate sperms were present. There were sperms with amorph head and giant free head lacking tails (**Figure 2a**).

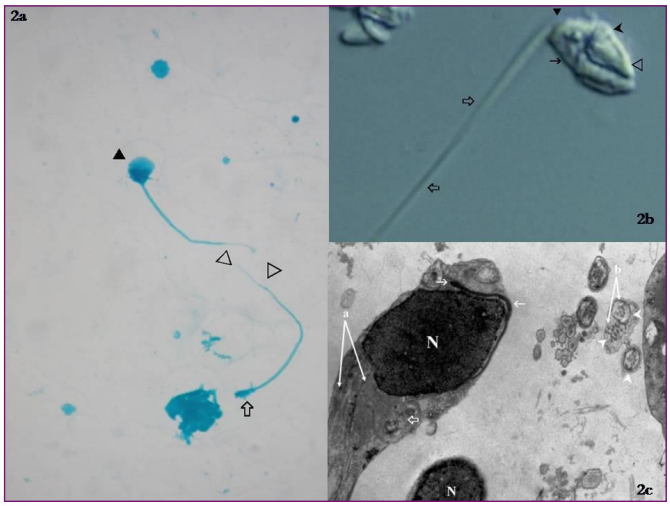

Figure 2. 2a. General appearance of Megalohead group: Megalohead sperms(⇒) were accompanied by pinhead sperms (▶). It's noticed that these sperms have tail abnormalities (□). Spermac Stain, x1000. **2b.** Sperm appearance in Megalohead group: Tail is not axially attached to the head (▶) and there are large residual cytoplasm (□). Straight tail structure is observed. (⇒). Abnormalities in acrosomal (□) and nuclear portions are noticed (▶). MSOME, x8050 2c. Sperm appearance in Megalohead group: Nucleus is large and includes mature chromatin (N). Acrosomal cap is observed in some areas of head but disrupted in some other areas (□). Residual cytoplasm is observed (⇒). There is deviation from 9+2 microtubule structure in axonemes. In longitudinal (a) and transversal (b) sections poliaxoneme structure is noticed (▶). Uranyl acetate- Lead citrate, x20000

Neck of the sperms evaluated by MSOME in the megalohead group weren't axially attached to head and were containing large residual cytoplasm. Mitochondrial distribution was irregular and there were multiple tails. Nuclei were usually amorph and configuration was distorted. High rate of vacuolization was observed in nuclear regions (**Figure 2b**).

In electron microscopic evaluation of this group nuclear structure was usually binuclear or large. Two nuclei not separated from each other in a single head was noteworthy. Acrosomal caps were in normal shape and were present in some parts of heads but on the other head disrupted in other parts with dense content. Residual cytoplasm was very frequently seen. Longitudinal and transverse sections revealed multiple tails. Deviation from 9 + 2 structure was seen in these axonemes. Mitochondrias were abnormal

in shape and Mitochondrial distribution was irregular (**Figure 2c**).

Globozoospermia Group

Light microscopic evaluation of this group revealed totally round heads without acrosomes. Mid piece was attached to the head axially and residual cytoplasm was noteworthy. Tails were straight, slender in the midportion, uncoiled and in normal length. Pinhead abnormality and amorph sperms were accompanying round head sperms. There were vacuoles in some of the round head sperms. Regional mitochondrial loss was seen in neck of most of the sperms. In another group of patients with type II defect there were acrososomal residues. Residual cytoplasm was rarely seen. Pinhead sperm abnormality was accompanying type II round sperms (**Figure 3a**).

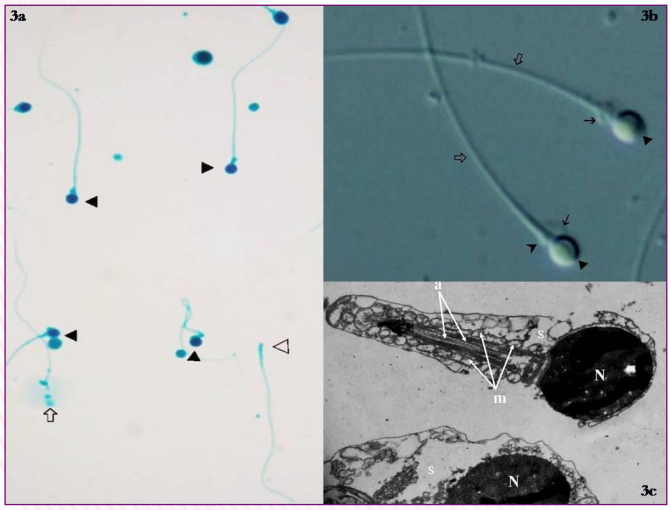

Figure 3. **3a**. General appearance of globozoospermia group: Evaluated sperm heads were totally round and were lacking acrosome (▶). Sperm with round head also accompanied by pinhead sperm abnormality (▽) and amorph sperms (⇒). Spermac Stain, x1000 **3b.** General appearance of globozoospermia group: Sperm heads were round and lacking acrosome (▶). Sperm neck contains residual cytoplasm (→), but there is no defect indicating lack of mitochondria or mitochondria disarrangement (➤). Tails are straight in shape, thin in the mid portion and uncoiled (⇒). MSOME, x8050 **3c.** Electron microscope appearance of globozoospermia group: Sperm nuclei are round and there is no acrosome (N). Axoneme structures are normal (a). Residual cytoplasm is observed in many sperms (s) Mitochondria are in normal shape and symmetrically located in both sides of neck (m). Uranyl acetate-Lead citrate, x20000

MSOME in Globozoospermia group has revealed sperm heads were round and lacking acrosomes. Neck was axially attached to the head and rarely contained residual cytoplasm. Mitochondria were regularly located in the neck region. However, in some sperms there were some disorderly conditions such as lacking or irregular mitochondria in the neck region. Tails were normal. In most of the sperm head a large and central vacuole was seen (**Figure 3b**).

Electron microscopic evaluation of Globozoospermia group has revealed round sperm heads without acrosomes. Nuclei were round and having condensed chromatin. Residual cytoplasm was very intense in sperms. In most of the sperm head a large and central vacuole was seen. Mitochondria were normal in shape and regularly and symmetrically located in both sides of the neck region. Axoneme structures were normal in shape, dynein arms and outer dense fibres were structurally normal. There sperms with normal microtubuler structure along with sperms deviating from 9+2 structure (**Figure 3c**).

Tail Stump Group

Evaluation of semen samples of this group revealed that some of the sperm heads were normal in shape but some other abnormal. However, abnormalities were seen in neck, mid piece and tail structures. Large residual cytoplasm was observed in some sperms. In many sperms tail was short and these were predominantly seen in a typical morphology which is short and thick tail. Free heads were frequently seen and rarely tails with normal length was also observed (**Figure 4a**).

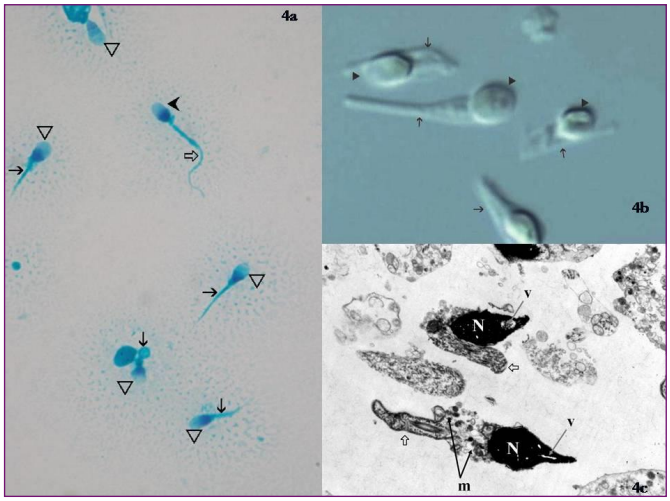

Figure 4. **4a.** General appearance of Tail stump group: Evaluated sperm heads are in normal (➤) and abnormal shape (▽). Tail morphology reveals usually a short tail (⇒), along with this, typical short thick tail shape of tail stump syndrome is noticeable (→). Spermac Stain, x1000 **4b.** General appearance of Tail stump group: In some evaluated patients sperm heads were found to be round (▶). Neck, mid-piece and tail characteristics of these sperms were observed to be abnormal. Accompanying short thick tail typical of tail stump syndrome is noticeable (→). MSOME, x8050 **4c.** Transmission electron microscope appearance of Tail stump group: Many of the evaluated nucleus were normal in shape, contained mature chromatin (N) and vacuoles (v). Dense and disarranged mitochondria were observed (m). Axoneme structures were found to be very short (⇒). Uranyl acetate-Lead citrate, x10000

Motility is meagre in tail stump syndrome thus using MSOME method in these patients is difficult. Thus, evaluation of these patients was only done by morphological analysis under higher magnification. MSOME has revealed acrosomes with normal structure in the sperm head and heads didn't contain vacuoles. Many necks were axially attached to the head and containing residual cytoplasm. There were sperms with free head in addition to sperms with short tails. Mitochondria were irregularly located within the sperm neck, there was lack of mitochondria in many sperm neck and there were disorders such as lacking or irregular mitochondria in the neck region. Some tails were attached to the head non-axially. In all tail stump samples sperm tails were short and these were predominantly seen in a typical morphology which is short and thick tail. Some sperms contained vacuoles and in some others sperm head was round and without acrosome (**Figure 4b**).

In the electron microscopic evaluation of tail stump group nuclei of sperms were normally shaped and having mature chromatin. In many sperms there wasn't any residual cytoplasm and mitochondria were intense and irregularly distributed. Fibrous sheath structure of sperms was disorderly and in pieces when it's available for evaluation. Acrosomal cap structure of many sperms was normal. Many axoneme structures were short. Observed

microtubule structures were irregularly distributed and having 9+0, 8+0 microtubule structure (**Figure 4c**).

Total Pinhead Group

In one of the patients in total pinhead group, sperm head couldn't be observed and in other patient only loosely attached sperm heads could be seen. There were variations in the neck, mid piece and tail parts of these sperms; however, they were usually structurally normal. Tail of sperms without head was straight. As a striking finding, there was residual cytoplasm at neck giving the impression of a head (**Figure 5a**).

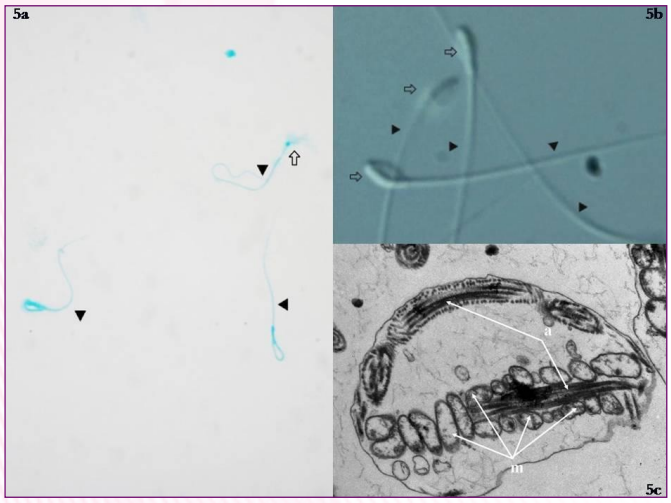

Figure 5: **5a.** General appearance of Total pinhead group: Sperms without head having tail that is straight, slender in the mid-portion, uncoiled and at normal length are observed. (▶). Residual cytoplasm is noticed at neck (⇒). Spermac stain, x1000 **5b.** General appearance of Total pinhead group: Sperms without head having straight tail structure (▶) and residual cytoplasm seems like head at neck region (⇒). MSOME, x8050 **5c**. Neck appearance in Total pinhead group: There is no nuclear content normal tail and several head ending, however axoneme structure is straight (a). Mitochondria are in straight and symmetrically located in both sides of neck (m). Uranyl acetate-Lead citrate, x30000

In MSOME there was sperm without head and with usually straight tail structure. Very rarely there were sperms with head non-axially and loosely attached to the neck. But in one patient residual cytoplasm giving the impression of a head at neck was noticeable (**Figure 5b**).

In the electron microscopic evaluation tail parts in longitudinal and transverse sections were noteworthy. Evaluated microtubular structure was 9+2. In patients normal tail structure occasionally ending with head was observed but there was no nuclear content. Mitochondria were symmetrically and regularly located within neck. Rarely in neck of some sperms there were findings relevant to lacking or irregular mitochondria. Axoneme structures were normal in general (**Figure 5c**).

DISCUSSION

Infertility is inability to conceive after 1 years of unprotected intercourse. Half of the issues involved with infertility are due to the man; but, in 30-50% etiology cannot be determined.[8] Currently semen analysis provides precious data relevant to male infertility and guidance for infertility treatment as the first test performed in male infertility.[9] Selection of a good-quality spermatozoon with normal morphology by using IMSI might be beneficial to embryonic development and to increase implantation and pregnancy rates. According to the majority of studies, it is not recommended to use IMSI routinely in the ART program. The couples with repeated implantation failures, patients with severe male factor infertility, advanced male and maternal ages are the populations who will have higher chances to conceive from IMSI. It is also recommended that diagnostic morphological evaluation of semen samples with high magnification is done before ICSI/IMSI procedure. Besides, according to the current knowledge, no prenatal or postnatal complications in the mothers and off- spring were reported following the IMSI procedure. The effectiveness of IMSI is still controversial mainly due to differences in inclusion criteria, stimulation protocols, seminal and oocyte qualities and many other confounding variables within the ART program. However, there is no doubt that the use of IMSI techniques can be helpful for some infertile couples to have a baby. [10]

It's known that morphological sperm abnormalities causing severe male infertility consist of megalohead, tail stump, Globozoospermia, total pinhead abnormality. The common result of ICSI procedure with these sperms are usually low fertilization rate, maldevelopment and/or late development of the fetus, high aneuploidy rate, low implantation and pregnancy rates. Since this precludes fertility substantially, it further decreases success rate of ICSI procedures performed on these patients.[2,3,11]

A well understood morphological pathology is important for guiding the treatment in centres using assisted reproductive method. However, there is no study evaluating all of the methods used for detecting above mentioned abnormalities at the same time. This study was designed to evaluate all of the methods used for detecting morphological sperm abnormalities causing severe male infertility at the same time, to evaluate relative superiority of these methods to each other and to answer the question of what's the best method to select the right sperm to increase chance of success before the ICSI procedure.

Currently WHO 2010 guideline is used as sperm morphology evaluation method; however, we used Kruger criteria in our study and evaluated sperm morphology accordingly.[7]

In the still most valid morphology evaluation, which is performed by Kruger et al.[13]; there should be a well-defined acrosomal region involving 40-70% of sperm head in the normal sperm. Mid piece should be long and slender and width should be less than 1 µm and its length should be 1.5X the head and it should be attached to the head axially. Residual cytoplasm may cover less than half of the normal head region. Tail should be straight, slender in the mid portion, uncoiled and nearly 45 µm long and at least 4% of the total sperms should be in accordance with this kind of sperm morphology. Sperm selection methods are an important challenge in assisted reproduction because most sperm characteristics cannot be tested, either in real time or in single cells referred to the ICSI procedure. Sperm selection under a magnification of ·400, in preparation for ICSI, allows the identification of major sperm morphological defects but does not provide information regarding the nuclear status of the sperm cell.[14]

In our study, sperm samples of fertile control group cases stained by Spermac stain were evaluated according to Kruger criteria and sperm morphology was found to be normal. Although there were some abnormal sperms, morphology of most of the sperms were normal or near normal.

In a study performed by obtaining images by digital imaging using invert light microscope under 6300x-8050x magnification by method described as MSOME, relationship between MSOME characteristics and ICSI outcome was assessed. Microinjection of sperms containing vacuoles was found to be correlated with early abortion and low pregnancy rates.6 In our Control group we observed that data obtained by MSOME was in accordance with the study of Bartoov et al.[6] Ability to use apparent normally shaped sperms is considered as a great advantage for ICSI procedure.

In publications studying ultra-structure of normal sperm in the usual description it was reported that in a single sperm totally enveloped by plasma membrane acrosomal cap is continuous and in normal position, shape, size and has intense content. It was observed that nucleus is in normal shape, has mature chromatin and sperm has no residual cytoplasm. Mitochondria is in normal shape and located regularly in both sides of the axoneme and there are nine peripheral microtubule pair along with two central microtubule structure (9×2+2).[15,16] In our electron microscopic evaluation we have observed that in the Control group acrosomal cap was in normal shape, continuous and its size was enough to involve 60% of sperm head from the anterior. Nucleus was in normal shape with mature chromatin and observed microtubule structures was 9+2. Mitochondria were in normal shape and located symmetrically in both sides of the neck. In our study we have observed that sperm ultra-structure was in accordance with the literature.

Megalohead described by Nistal et al.[17] is a very special form of sperm morphologic defects and is characterized by presence of low motility sperms, varying number of tail structure, irregular large sized heads. The same group have also reported that in cases having pinhead abnormality other morphological sperm abnormalities were also seen in varying proportions.

Kahraman et al.[11] have reported in their study that in megalohead sperm samples the most important morphological features are sperm with multiple heads and without tail and substantial amount of immature spermatid in ejaculate/testicular sperm extraction solution.

General evaluation of semen samples obtained from patients with megalohead sperm multiple tails, irregular large sized sperm heads, sperms with pinhead abnormality in most of the samples, amorph and irregular head shape along with acrosomal cysts in the acrosomal region were observed. In MSOME of megalohead group in our study nucleus was usually large, amorph and with distorted configuration and lots of sperms containing vacuoles were observed. In our study MSOME results were similar with the results of light microscopic examination. We suggest that the ability to use sperms near normal size observed in MSOME may provide a great advantage.

The high magnification approach is also of particular benefit when used in situations in which the identification of specific sperm organelles is required, such as the acrosomal components in cases of globozoospermia. Sermondade et al.[18] reported a successful pregnancy and healthy childbirth in a case of total globozoospermia after IMSI.

Chelli et al.[19] studied the chromosomal content of spermatozoa selected by IMSI in two cases of macrocephalic sperm head syndrome. FISH was performed in selected spermatozoa with normal-sized heads after IMSI selection. However, of the six spermatozoa that could be selected, all were aneuploid.[14]

Escalier has described a quantitative morphology for megalohead and multiple tailed sperm cells in 1983 by studying 6 infertile men.[20] In other electron microscopic studies about megalohead sperm cells several nuclei could be clearly distinguished within the head and it was suggested that this may occur due to defects arising during spermiogenesis I or spermatogenesis II stage of meiosis.[11] In our study, it was observed that in general, nuclear structure was double and large. Multiple tail structure is observed rather in longitudinal and transverse sections and deviation from 9+2 microrubule structure was observed in these axonemes. It was determined that results of our electron microscopic evaluation were in accordance with the literature.

In the study performed by Singh et al.[21] two sub types of Globozoospermia was described. Type I Globozoospermia is described as absolute absence of acrosom content. They reported lack of acrosome along with structural defects in axoneme, tail, mitochondria and microtubules. They have stated that in type II Globozoospermia acrosome residues were observed and there was a conic nucleus with surrounding cytoplasmic ringlets.[2,3] In our study, in type I Globozoospermia group it was observed that sperm heads were totally round and there was lack of acrosome in the head region. It was observed that residual cytoplasm was prominent and pinhead abnormality and amorph sperms were also observed. In type II Globozoospermia conic nucleus and a small amount of acrosome within the sperm was noteworthy. When evaluated by MSOME, in Globozoospermiaa sperm heads were round and lacking acrosome and large vacuoles observed in Globozoospermia were interpreted as an indicator of DNA damage in nuclear region. Bendayan et al.[22] demonstrated that sperm with vacuoles carry altered epigenetic marks (H3K4me3, H3K27me3) compared to sperm without vacuoles. MSOME/IMSI could be the first tool in assisted reproduction capable of reducing the risk of transmission of epigenetic problems through spermatozoa.[23]

It was reported that in type I Globozoospermiaa chromatin has spherical distribution and there is absolute absence of acrosome and there were structural defects in axoneme, tail, mitochondria and microtubules along with large vacuoles in the sperm head.[21] Electron microscopic evaluation of Globozoospermiaa group revealed lack of acrosome in the sperm head and a round and central vacuole. There were microtubules in line with 9+2 structure or deviating from 9+2 structure in compliance with previous studies.[24]

Chemes et al.[25,26] have described tail stump sperms and reported that they were totally immobile and were characterized by severe problems in sperm fibrous sheath organization. Tail is particularly short and thick thus it's called tail stump syndrome.[26] In our general evaluation of tail stump group, we have observed short tail along with short and thick tail structures. There were free heads and rarely tail with normal size. When MSOME was done in tail stump group it was observed that motile sperm percentage was low; thus morphological evaluation was performed under greater magnification and it was seen that tails were usually short and thick. In our study similar findings were observed when light microscope was used. However, it was considered that to select sperm with near normal long tail by Intracytoplasmic Morphologically *selected Sperm* Injection (*IMSI*) may be the method of choice.

Fibrous sheath dysplasia or tail stump syndrome was described in 6 different types in 2006 by Mitchell et al.[27] In electron microscopic evaluation of tail stump group in our study, it was observed that fibrous sheath structure was continuous in some regions on the other hand it's defective and in pieces in some other regions. Microtubule structures were deviating from 9+2 structure (9+0, 8+0) and microtubule structures were located in a disorganized way.

Perotti et al.[28] have stated that in an infertile patient most of the sperms didn't have any head and heads were detached. In the evaluation of total pinhead group in our study, we have observed sperms without head region but containing intense residual cytoplasm in the neck region. Additionally, there were sperms with head, though very rare. By MSOME in total pinhead group there were sperms with straight tail structure but without head and rarely sperms with head attached loosely to neck region. In our study similar findings were observed when light microscope was used. It was concluded that ability to use sperms with apparent head may create great advantage.

Chemes et al.[29] have shown in their study that pinhead sperms occur because of disorders related with midpiece attachment structure. Electron microscopic evaluation in our study revealed tail pieces in longitudinal and transverse sections. Observed microtubuler structures were in 9+2 structure. Sperm selection with MSOME parameters and IMSI can improve the embryo quality that was aimed using TLM and clinical outcomes in couples with male factor infertility. The data confirmed that patients with OAT and T took the most benefits from these advanced imaging systems of MSOME and TLM.[30]

There is still no consensus regarding the clinical value of IMSI. The contradictory results are caused by the lack of standardization of microscopy techniques used for ICSI and IMSI. Comparisons between IMSI and ICSI fertilization cycles may yield different results depending on the microscope setups used for ICSI and IMSI. When a high-quality system is used for ICSI that allows for visualizations of sperm vacuoles and obtained results are compared with IMSI, there may not be an apparent improvement. However, the clinics with a less advanced ICSI setup may experience improvements with the use of IMSI. This is why it is crucial to know what is being compared. In order to assess the influence of sperm vacuoles, the first step should be to set up the ICSI and IMSI systems and determine the size of vacuoles that can be visualized with each system.[31]

In our study; in addition to general morphological evaluation used in sperm defects causing severe male infertility we used MSOME for real time evaluation of sperm morphology and electron microscope and all of the methods gave results similar to previous studies irrespective of the evaluated groups and each evaluation method supported each other in respect of morphological evaluation findings. While it is thought that the selection of the best vacuoleless sperm at high magnifications, which was the opinion in the first years of the IMSI technique, can increase the success of the ICSI technique; We were of the opinion that the success rates of the samples with sperm morphological features that cause severe male infertility, which were examined within the scope of our study, could increase when the IMSI technique is used. The studies conducted so far support the initial views and results of our thesis. In addition, our study is the only IMSI study that examines sperm morphologies that cause severe male infertility.

Study Limitations: In our study, sperm morphologies that cause severe male infertility were examined, and no information was given about the embryo development and pregnancies of these patients.

CONCLUSION

As a conclusion, evaluating sperm morphology with MSOME which is a novel method in addition to light and electron microscopic methods may provide sufficient and important information in a short time. Also, in severe male infertility use of ICSI method developed by using sperms selected by this method may make significant contribution to success rate of centres using assisted reproductive method.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study is the scientific study of Ferhat Cengiz master's thesis named "Morphological analysis of sperms causing severe male infertility with light microscopy, motile sperm organellar morphology examination and electron microscopy technics", registered at the National Thesis Center with the number 267264, dated 19.07.2010.

Informed Consent: All patients signed the free and informed consent form.

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